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Stimulation of Immunoreactive Insulin Release by Glucose in Rat Brain Synaptosomes

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The effect of glucose on the release of immunoreactive insulin (IRI) in synaptosomes isolated from rat brain was studied. In the absence of glucose synaptosomes release about 4% (0.77 μIU/mg protein) of total content. Glucose increases significantly the IRI released by synaptosomes. Addition of the glycolytic inhibitor iodoacetic acid (IAA), decreased the glucose-induced release of IRI by about 50%, suggesting that glucose metabolism is involved. The observation that glucose provides a concentration related signal for IRI release indicates that this synaptosomal preparation may be useful as a model for research on the mechanism of insulin release in brain.

KEY WORDS: Synaptosomes; glucose; brain insulin; insulin release.

INTRODUCTION

Under normal physiological conditions, glucose is the most important source of energy utilized by brain. Besides yielding ATP, glucose metabolism contributes to the synthesis of the neurotransmitters acetylcholine, glutamate, aspartate, GABA and glycine. Perturbations in the supply of glucose, as well as impairments of glucose metabolism, modify brain function (1). The glucose transport from blood into the brain is mediated by at least two glucose transporter proteins, GLUT1 and GLUT3 (2). It was proposed that up regulation of expression of GLUT3, which is present predominantly in neurons, under glucose deprivation or ischaemic insult, represents a protective mechanism against energy depletion in neurons (3-6).

Presently, there is a great deal of evidence that glycolytic flux and oxidative metabolism in brain are stimulated by insulin indicating that the control of glucose metabolism by insulin is similar to that observed in non-neuronal tissues (7–9). On the other hand, the existence of both insulin and insulin receptors in brain is well established (10,11). Most of brain insulin is assumed to be transported from the periphery, but at present there is increasing evidence that insulin or insulin-like peptide can also be synthesized by neurons in high localized areas of the brain (12).

The presence of insulin, insulin receptors and glucose transporters in brain makes it likely that neurons may respond to glucose concentration by releasing insulin. Since synaptosomes isolated from rat brain store IRI in synaptic vesicles, from which it can be released by exocytosis (13), we studied and reported here the release of IRI in response to glucose.

EXPERIMENTAL PROCEDURE

Preparation of Synaptosomes. Crude synaptosomes were prepared from brain of male Wistar rats (180–220 g), according to the method of Hajos (24), with some modifications. In brief, the whole brain was homogenized in 10 volumes of 0.32 M sucrose buffered at pH 7.4 with Tris. The homogenate was centrifuged at 1,000 *g* for 10

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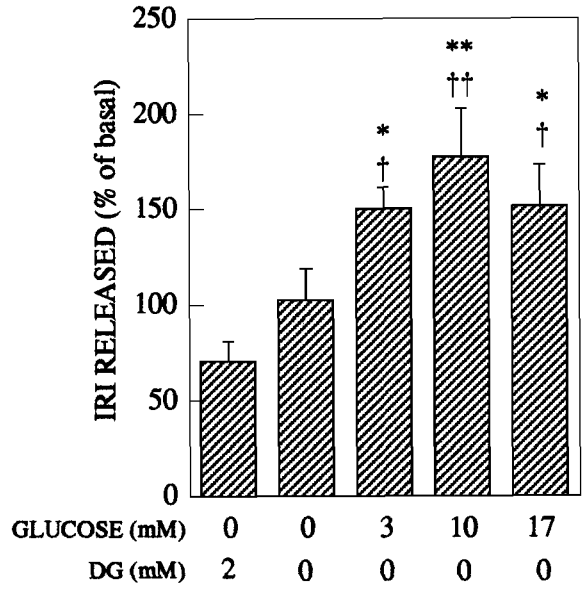

Fig. 1. Effect of glucose on the release of immunoreactive insulin (IRI) in synaptosomes. Synaptosomes were incubated for 10 min at 30 °C as described in Experimental Procedure. IRI data, expressed as a percentage of basal release observed in the absence of glucose, are mean ± SEM from 12 experiments except the DG experiments which are n = 5. *p < 0.05, **p < 0.001, as compared with the release in the absence of glucose; †p < 0.01, ††p < 0.001, as compared with the release in the presence of DG in glucose-free medium. IRI release in 0 mM glucose was 0.77 ± 0.17 μIU/mg protein/10 min.

Table I. Immunoreactive Insulin (IRI) Content in Brain and Synaptosomal Preparations

	IRI content
Whole rat brain	5.68 ± 0.32 μUI/mg protein (n = 5)
Synaptosomal preparation	21.82 ± 3.67 μUI/mg protein (n = 11)

IRI content was determined by the radioimmunoassay technic. Conditions of analysis are described in Experimental Procedure. Results are the mean ± SEM for the number (n) of experiments indicated.

min and the synaptosomes were isolated from the supernatant by centrifugation at 12,000 *g* for 20 min. The white and fluffy synaptosome layer was then resuspended, respun and resuspended in the sucrose medium at a protein concentration of 15–20 mg/ml, as determined by the biuret method. Experiments were carried out within 2 h of preparation.

Incubation Conditions. Synaptosomes were incubated at a concentration of 3–4 mg protein/ml, for 10 min at 30 °C in a standard medium containing (in mM): 118 NaCl, 3 KCl, 1.2 $MgCl_2$, 1 NaH_2PO_4, 1.2 $CaCl_2$, 10 Hepes adjusted to pH 7.4 with Tris, 0.1% of bovine serum albumin and various concentrations of glucose. The incubation was terminated by centrifugation (120 s in a Eppendorf microcentrifuge), and the supernatant was radioimmunoassayed for IRI. The total radioimmunoactive insulin in synaptosomes was measured according to the method described by Tesoriere et al. (25). The synaptosomes were lysed with water (1 ml/3–4 mg protein) and sonicated for 30 sec (3 pulses of 10 sec). The homogenates were centrifuged at 20,000 *g* for 10 min and the supernatants assayed. IRI extracts from whole rat brain were prepared by the method of Havrankova et al. (14).

Conditions of low metabolism were induced in synaptosomes incubated in the presence of chemical agents, as previously described (17): (1) 2 mM KCN + 5 μg/ml oligomycin (2) 2 mM IAA and (3) 2 mM DG. KCN inhibits the respiratory chain by inhibiting cytochrome c oxidase, oligomycin inhibits the ATP synthase, IAA, which inhibits the glycolytic enzyme glyceraldehyde dehydrogenase and DG which substitutes glucose in the medium and is not metabolized by the cells inhibiting competitively glycolysis and glycogenolysis. Test compounds were introduced into the incubation medium before the addition of protein. In experiments where glucose was omitted, NaCl was raised to 123 mM.

Measurement of Immunoreactive Insulin (IRI) in Brain and Synaptosomes. Immunoreactive insulin was measured by using the Coat-A-Count radioimmunoassay insulin assay kit (Diagnostic Products Corporation, Los Angeles CA, USA).

Statistics. Statistical analysis of data was carried out by using the one-factor ANOVA with a Fisher's PLSD post-test for multiple comparisons. The means ± SEM are presented and differences with a P value < 0.05 were considered significant.

RESULTS

Data presented in Fig. 1 show that glucose increased the release of IRI in rat brain synatosomes. The presence of 17 mM of glucose also elicited IRI release but the amount of IRI released did not differ significantly from that seen with 3 or 10 mM glucose. The released IRI at 0 mM glucose in the medium (0.77 ± 0.17 μUI/mg protein represented about 4% of the total synaptosomal IRI content (21.82 ± 3.67 μUI/mg protein, Table I). In comparison with whole brain extracts, which contains 5.68 ± 0.32 μUI/mg protein (134.9 ± 18.2 μUI/g wet brain), synaptosomes were enriched about 4 fold (Table I).

To determine whether glucose-induced IRI release was related to glycolysis, we tested the effect of IAA and DG, two known inhibitors of glycolysis. As shown in Fig. 1, DG significantly reduced the release of IRI by about 30% in synaptosomes incubated in the absence of glucose. Table II shows the release of IRI from synaptosomes incubated in the presence of 17 mM glucose. The addition of 2 mM of IAA into the assay medium caused a significant decrease ($p < 0.05$), in the IRI level as compared to control synaptosomes. The amount of IRI release under hypoxic-like conditions, when oxidative metabolism is inhibited by KCN + olygomycin, was increased by about 35% when compared with the IRI released in control conditions (Table II).

Table II. IRI Release in Synaptosomes Incubated Under Conditions of Low Metabolism

	IRI (% of control)
Control	100 ± 10.73 (n = 8)
KCN + Oligomycin	134.66 ± 18.27* (n = 8)
IAA	45.88 ± 6.71**/† (n = 5)

Synaptosomes were incubated for 10 min in the presence of 17 mM glucose. The conditions of low metabolism were induced with iodoacetic acid (IAA) or potassium cyanide (KCN) + oligomycin. * $p < 0.05$, ** $p < 0.01$, as compared to the data in control conditions; †$p < 0.001$ as compared to the data obtained in the presence of KCN + oligomycin. The value of release of IRI in the presence of glucose 17 mM was 1.11 ± 0.17 μIU/mg protein/10 min. Results are the mean ± SEM of the number (n) of experiments indicated.

DISCUSSION

The results reported in this paper show that IRI is present in rat brain synaptosomes and that glucose causes an enhancement of the IRI release. The level of whole rat brain IRI determined in our study is well in accord with the values reported by various laboratories (10,13,14). If 1 g insulin contains 25 IU of bioactivity, the total whole brain extract contains about 5 ng IRI per g wet brain. This value is near the mean values previously reported (3–9 ng/g, 10, 13). However, the content in IRI of our synaptosomal preparation (0.873 ng/mg protein), is fivefold higher than that observed by others in purified synaptosomes (0.185 ng/mg protein) (13). These differences are probably due to different isolation methods and/or to different extraction procedures.

In pancreatic β-cells the rate of glucose metabolism is the major determinant of insulin secretion (15). We found that the release of IRI was significantly enhanced when synaptosomes were exposed to increasing concentrations of glucose (Fig. 1), suggesting that the release of IRI in brain is regulated by glucose metabolism. When DG or IAA was added to the reaction medium the IRI release was inhibited. This finding shows that the effect of glucose in IRI is exerted through glycolysis. Additionally, insulin release from rat brain neuronal cells in culture and synaptosomal preparations was also observed following depolarization with veratridine or K^+ in the presence of Ca^{2+} (13,16), suggesting that other mechanism(s) are involved in the IRI release in brain.

The lower release produced by DG or IAA than by glucose deprivation is probably explained by the presence of endogenous substrates that can sustain glycolysis in the absence of glucose. Inhibition with glycolytic inhibitors produces a complete glycolytic blockade (17). Glycolysis plays a very important role in regulating energy status in nerve terminals (18). The increase in IRI observed in synaptosomes under anoxia-like conditions suggests that under anoxia glycolysis may be stimulated. The stimulation of glycolysis was already proposed to occur in synaptosomes, upon inhibition of the respiratory chain by cyanide (18).

Thus glucose metabolism may provide a signal for insulin release in the brain. Interestingly, a beneficial effect of insulin in reducing cerebral brain damage during several stress conditions, such as ischaemia and traumatic brain injury was recently demonstrated, suggesting that insulin could be neuroprotective *per si* (19–21). Desensitization of the neuronal insulin receptors and disturbances in glucose metabolism are hypothesized to be a cause of the age-associated neurodegenerative disorders like in Alzheimer and Parkinson's disease (22,23). At present the exact mechanism by which glucose leads to increase in IRI in brain is not known. In this regard, we are investigating the involvement of K^+ATP channels, plasma membrane potential, Ca^{2+} influx and changes in ATP or ATP/ADP ratio on the glucose-induced IRI release.

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REFERENCES

1. Nehlig, A. 1997. Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycaemia. Diabetes and Metab. 23:18–29.
2. Pressin, J. E. and Bell, G. I. 1992. Mammalian facilitative glucose transporter family: structure and molecular regulation Annu. Rev. Physiol. 54:911–930.
3. Lee, H. T. and Bondy, C. A. 1993. Ischemic injury induces brain glucose transporter gene expression. Endocrinology 133:2540–2544.
4. Gerhart, D. Z., Leino, R. L., Taylor, W. E., Borson, N. D., and Drewes, L. R. 1994. GLUT1 and GLU3 gene expression in gerbil brain following brief ischemia-an in situ hybridization study. Brain Res. 25:313-322.
5. Nagamatsu, S., Sawa, H., Inoue, N., Nakamichi, Y., Takeshima, H., and Hoshino, T. 1994. Gene expression of GLU3 glucose transporter regulated by glucose *in vivo* in mouse brain and *in vitro* in neuronal cell cultures from rat brain. Biochem. J. 300: 125–131.
6. Vannucci, S. 1994. Developmental expression of GLUT1 and GLUT3 glucose transporters in rat brain. J. Neurochem. 62:240–246.
7. Henneberg, N. and Hoyer, S. 1994. Short-term or long-term intracerebroventricular (i.c.v.) infusion of insulin exhibits a discrete anabolic effect on cerebral energy metabolism in the rat. Neurosc. Lett. 175:153–156.
8. Hoyer, S., Prem, L., Sorbi, S., and Amaducci, L. 1993. Stimulation of glycolytic key enzymes in cerebral cortex by insulin. NeuroReport 4:991–993.

9. Schwartz, M. W., Figlewicz, D., P., Baskin, D. G., Woods, S. C., and Porte, D. 1992. Insulin in the brain: a hormonal regulator of energy metabolism. Endocrine Rev. 13:387-414.
10. Baskin, D. G., Wilcox, B. J., Figlewicz, D. P., and Dorsa, D. M. 1988. Insulin and insulin-like growth factors in the CNS. TINS 11:107–111.
11. Wozniak, M., Rydzewski, B., Baker, S. P. and Raizada, M. K. 1993. The cellular and physiological actions of insulin in the central nervous system. Neurochem. Int. 22:1–10.
12. Devaskar, S. U., Giddings, S. J., Rajakumar, P. A., Carnaghi, L. R., Menon, R. K., and Zahm, D. S. 1994. Insulin gene expression and insulin synthesis in mammalian neuronal cells. J. Biol. Chem. 269:8445-8454.
13. Wei, L., Matsumoto, H., and Rhoads, D. E. 1990. Release of immunoreactive insulin from rat brain synaptosomes under depolarizing conditions. J. Neurochem. 54:1661–1665.
14. Havrankova, J., Schmechel, D., Roth, J., and Brownstein, M. 1978. Identification of insulin in rat brain. Proc. Natl. Acad. Sci. USA. 75:5337–5741.
15. Hedeskov, C. J. 1980. Mechanism of glucose-induced insulin secretion. Physiol. Reviews 60:442–509.
16. Clarke, D. W., Mudd, L., Boyd, F., Fields, M., and Raizada, M. K. 1986. Insulin is released from rat brain neuronal cells in culture. J. Neurochem. 47:831-836.
17. Swanson, R. A., Yu, A. C. H., Sharp, F. R., and Chan, P. H. 1989. Regulation of glycogen content in primary astrocyte culture: effects of glucose analogues, phenobarbital, and methionine sulfoximine. J. Neurochem. 52:1359–1365.
18. Kauppinen, R. A. and Nicholls, D. G. 1986. Failure to maintain glycolysis in anoxic nerve terminals. J. Neurochem. 47:1864–1869.
19. Lemay, D. R., Gehau, L. R., Zelenock, G. B., and D'Alecy, L. G. 1988. Insulin administration protects neurological function in cerebral ischemia in rats. Stroke 19:1411–1419.
20. Strong, A. J., Fairfield, J. E., Monteiro, E., Kirby, M., Hogg, A. R., Shape, M., and Rossfield, L. 1990. J. Neurol. Neurosurg. Psychiatry 53:847–853.
21. Zhu, C. Z. and Auer, R. N. 1994. Intraventricular administration of insulin and IGF-1 in transient forebrain ischemia. J. Cereb. Blood Flow Metab. 14:237–242.
22. Degen, D. B., Frolich, L., Hoyer, S. and Riederer, P. 1995. Altered regulation of brain glucose metabolism as a cause of neurodegenerative disorders. J. Neural. Transm. 46:139–147.
23. Hoyer, S. 1996. Oxidative metabolism deficiencies in brains of patients with Alzheimer's disease. Acta Neurol. Scand. 165:18–24.
24. Hajos, F. 1975. An improved method for the preparation of synaptosomal fractions in high purity. Brain Res. 93:485-499.
25. Tesoriere, G., Vento, R., Calvaruso, G., Taibi, G., and Giuliano, M. 1992. Identification of insulin in chick embryo retina during development and its inhibitory effect on DNA synthesis. J. Neurochem. 58:1353-1359.
26. Santos, M. S., Moreno, A. J., and Carvalho, A. P. 1996. Relationships between ATP depletion, membrane potential, and the release of neurotransmitters in rat nerve terminals: an in vitro study under conditions that mimic anoxia, hypoglycemia and ischemia. Stroke 27:941-970.